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IS 11301 (1985): Methods for determination of phosalone residues in food commodities [FAD 1: Pesticides and Pesticides Residue Analysis]



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(Reaffirmed 2000)

Indian Standard

**METHODS FOR
DETERMINATION OF PHOSALONE RESIDUES
IN FOOD COMMODITIES**

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INDIAN STANDARDS INSTITUTION
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

Indian Standard

METHODS FOR DETERMINATION OF PHOSALONE RESIDUES IN FOOD COMMODITIES

Pesticides Residue Analysis Sectional Committee, AFDC 56

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Bangalow **No. A**, Malkaganj
Delhi

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National Organic Chemical Industries Ltd,
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Central Food Laboratory, Calcutta; and Central
Committee for Food Standards. New Delhi

Directorate of Plant Protection, Quarantine &
Storage (Ministry of Agriculture & Rural
Development), Faridabad

Bayer India Ltd, Thane

Department of Entomology, Punjab Agricultural
University, **Ludhiana**

C, S. A. University of Agriculture & Technology,
Kanpui

Department of Food (Ministry of Agriculture &
Rural Development), New Delhi

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Indian Standard

METHODS FOR DETERMINATION OF PHOSALONE RESIDUES IN FOOD COMMODITIES

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 24 June 1985, after the draft finalized by the Pesticides Residues Analysis Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Phosalone formulations are used in agriculture for the control of many insect pests. Frequent and increased use of these formulations often result in harmful effects due to toxic nature of residues. Careful monitoring of residues, is therefore, an important step in safeguarding human health and in formulation of regulatory policy.

0.3 In the preparation of this standard due consideration has been given to the limits likely to be laid down under the provisions of Prevention of Food Adulteration Rules and the specified test methods are sensitive to the levels indicated in **1.1.1**.

0.4 This standard will enable the health authorities and other engaged in the field to follow a uniform test procedure **for** the estimation of phosalone residues in various foods.

0.5 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed **or** calculated, is to be rounded off, it shall be done in accordance with IS :2-1960*.

1. SCOPE

1.1 This standard prescribes gas chromatographic (GLC) methods using alkali flame-ionization detector (AFID), and electron capture detector (ECD) for the determination of phosalone [0, *O*-diethyl-*S*-(6-chlorobenzoxazolone-3-yl)-methyl phosphorodithioate] residues in food commodities.

*Rules for rounding off numerical values (*revised*).

1.1.1 The minimum limit of determination of phosalone residues using alkali flame-ionization detector is **0.1 µg/g** while it is **0.05 µg/g** with electron capture detector.

2. QUALITY OF REAGENTS

2.1 Unless specified otherwise, pure chemicals and distilled water (see IS :**1070-1977***) shall *be* employed in tests.

NOTE -- 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the result of analysis,

3. SAMPLING

3.1 Representative samples of the material for estimating phosalone residues shall be drawn in accordance with IS : 11380 (Part 1)-1985†.

4. EXTRACTION

4.1 Reagents

4.1.1 Solvents — Acetone, **acetonitrile**, petroleum ether, n-hexane and dichloromethane. All glass distilled.

4.1.2 Sodium Chloride

4.1.3 Sodium Sulphate— Anhydrous.

4.1.4 Glass Wool — Rinse with acetone and methanol several times and dry before use.

4.1.5 Adsorbent for Column Chromatography — Florisil (60 to 100 mesh).

4.2 Apparatus

4.2.1 High Speed Blender — Waring or equivalent with glass jar, teflon washers and seals.

4.2.2 Centrifuge — Capable of 3 000 r/min and having proper capacity centrifuge bottles.

4.2.3 Kuderna-Danish Concentrator -Having 500-ml flask and 10 to 15-ml receiving tube with Snyder column.

4.2.4 Rotary Vacuum Evaporator

*Specification for water for general laboratory use (second revision).

†Methods of sampling for the determination of pesticide residues: Part I Agricultural and food commodities.

4.2.5 Separating Funnel — one-litre capacity.

4.2.6 Chromatographic Tube — 2.2 cm outer diameter and 40 cm long with stop-cock.

4.3 Non-fatty Foods (Less Than 2 Percent Fat Content)

4.3.1 Vegetables and Fruits (Containing More Than 75 Percent Moisture) — Transfer 50 g of the sample to a **waring** blender with 100 ml acetone and blend for 2 minutes at high speed. Filter extract through pre-washed glass wool plug held on a conical funnel into a beaker. Re-extract the residual sample material with two 50 ml portions of acetone by blending for 2 minutes each and filter through the same glass **wool** plug. Concentrate the combined extract to about 100 ml in a rotary vacuum evaporator. Transfer it to a 1-litre separating funnel, containing 100 ml petroleum ether and 100 ml of dichloromethane. Shake for 1 minute, allow the layers to separate. Transfer the lower aqueous layer into another **1-litre** separating funnel. Dry the retained upper organic layer of the first separating funnel by passing through 4 cm thick anhydrous sodium sulphate supported on glass wool in a funnel and collect in a Kuderna-Danish concentrator or rotary vacuum evaporator flask. To the separating funnel containing the aqueous phase, add 5 g sodium chloride and shake for half-a-minute to dissolve the salt. Add 100 ml dichloromethane, shake for one minute and allow the phases to separate. Collect the lower organic phase into the Kuderna-Danish concentrator after passing it through the same anhydrous sodium sulphate plug. Repeat partitioning with one more portion of 100 ml of dichloromethane. Rinse the sodium sulphate plug with 50 ml of dichloromethane into the concentrator. Discard the aqueous fraction. Evaporate the combined partitioned organic extract in a Kuderna-Danish concentrator/rotary vacuum evaporator to suitable volume. Ensure complete removal of dichloromethane by repeated evaporation with the addition of 20 ml acetone. Concentrate to about 2 ml.

4.3.2 Cereals and Pulses (Containing Less Than 20 Percent Moisture) — Weigh 20.0 g of the sample into the blender jar and add 150 ml of 35 percent water in acetone (350 ml distilled water diluted to 1 litre with acetone). Blend at high speed for 2 minutes and filter under pressure through pre-washed **Whatman** filter paper No. 1 placed on a Buchner funnel. Re-extract the residual material with two 50 ml portions of 35 percent water-acetone by blending for 2 minutes and filter under pressure quickly. Concentrate the combined filtrate to about 100 ml in a rotary vacuum evaporator/Kuderna-Danish concentrator and transfer to a **1-litre** separating funnel containing 100 ml of dichloromethane and 100 ml of petroleum ether. Partition and concentrate to volume as in 4.3.1.

4.3.3 Water and Beverages — Partition twice 500 ml portion of 1-litre sample having 7 g sodium chloride dissolved in it with 100 ml of dichloromethane in a separating funnel. Collect the lower dichloromethane layer through a plug of anhydrous sodium sulphate and concentrate as described in 4.3.1.

4.4 Fatty Foods (Containing More Than 2 Percent Fat)

4.4.1 Fats and Oils — Completely dissolve 5 g **sample** in about 150 ml of n-hexane and transfer to a separating funnel. Partition thrice with 100, 50, 50 ml of acetonitrile. Each time collecting the lower acetonitrile layer into the second separating funnel, containing 600 ml of 2 percent sodium **chloride** solution in distilled water. To this, add 100 ml of petroleum ether and shake thoroughly for 1 minute and allow layers to separate. Drain the lower aqueous phase into another separating funnel and repeat partitioning twice more. Combine the upper petroleum ether layer by passing over a plug of anhydrous sodium sulphate and concentrate in a rotary vacuum evaporator/Kuderna-Danish concentrator to about 5 ml.

4.4.2 Oilseeds and Nuts-Transfer 50.0 g of crushed sample with 10 g anhydrous sodium sulphate into a blender jar. Add 150 ml of acetone and blend at high speed for 3 minutes. Filter under pressure through a pre-washed **Whatman** No. 1 filter paper placed over a Buchner funnel. Re-extract the residual material twice more with 75 ml portion of acetone. Evaporate the combined filtrate to almost oily layer in a Kuderna-Danish-Concentrator/rotary vacuum evaporator. Dissolve oily residue in 100 ml of n-hexane and partition with acetonitrile as in 4.4.1.

4.4.3 Meat and Fish — Transfer 50.0 g of the minced sample with 20 g of anhydrous sodium sulphate in a blender jar. Add 150 ml of n-hexane-ketone (2 : 1 v/v) and blend for 3 minutes at high speed. Filter under pressure through prewashed **Whatman** filter paper No. 1 placed on a Buchner funnel. Re-extract the residual material twice with 100 ml portion of n-hexane-acetone and follow concentration and partitioning as given in 4.4.1.

4.4.4 Milk and Milk Products-Transfer 100 g milk in the blender jar. Add 10 g of anhydrous sodium sulphate and 100 ml of acetone and blend for 2 minutes. Filter under pressure through prewashed **Whatman** filter paper No. 1 placed on a Buchner funnel. Re-extract the residual material with 100 ml of acetone. Follow steps as in 4.4.1 after concentration to oily layer.

NOTE 1 — As the extraction and partitioning procedures are based on **fat, moisture, and sugar content of the food material, refer food composition tables for these values given in The Nutrition Value of Indian Foods and Planning of Satisfactory Diets by W. R. Aykroyd, ICMR, New Delhi or equivalent.**

NOTE 2 -Where sample extracts pose difficulty in filtration or thick emulsions are formed during partitioning, centrifugation of the material at about 3 000 r/min for about 10 minutes can be resorted to.

4.5 Clean Up

4.5.1 The partitioned extracts obtained in 4.3.1 to 4.3.3 are suitable for direct estimation by alkali flame ionization detector up to $0.1 \mu\text{g/g}$ level. However, for extracts from 4.4.1 to 4.4.4 and estimations below $0.1 \mu\text{g/g}$ level of the residues and/or estimation by electron capture detector, further clean up by column chromatography is necessary.

4.5.2 Prepare the chromatographic column by packing with 1 cm layer of glass wool at the bottom followed by 10 cm layer of florisil and 1 cm layer of anhydrous sodium sulphate. Prewash this column with 100 ml of benzene and discard eluate. Dissolve residues in the concentrator tube/flask in two 5 ml portions of benzene and transfer to the column bed. Open the stopcock and allow the column to run till the transferred material soaks into the absorbent. Close the stopcock and fill the column with 200 ml of elutant, benzene-ethyl acetate solution (95 : 5 v/v). Collect in a round bottomed flask. Concentrate eluate in a rotary vacuum evaporator on water bath at temperature of 45°C . Transfer quantitatively to a graduated test tube with acetone and make to suitable volume for analysis.

4.5.3 Calibrate the Aorisil column by eluting standard solution of ($1 \mu\text{g/ml}$) phosalone with the elutant.

5. GAS CHROMATOGRAPHIC DETERMINATION

5.1 Principle — Phosalone residues obtained after sample extraction and partitioning are measured by gas chromatograph equipped with alkali flame ionization detector (AFID). Quantitation by gas chromatograph equipped with electron capture detector (ECD) require further clean-up by column chromatography. The amount of residues is determined by comparing the response with the response for a known standard of similar concentration.

5.2 Apparatus

5.2.1 Gas Chromatograph — A gas chromatograph equipped with thermoionic detector operated under the following suggested parameters. These parameters may be varied as per available facilities provided standardization is done:

Column

Glass, 1 meter long and 3 mm i.d. packed with 3 percent DC-200 on 80 to 100 mesh Gas chrom Q.

Injection port temperature	220°C
Column oven temperature	230°C
Detector temperature	240°C
Carrier gas	Nitrogen 70 ml/min
	Hydrogen 40 ml/min
	Air 400 ml/min
Attenuation	1×10^{-9} to 23×10^{-9} A
Recorder chart flow	2.5 cm/min

5.2.2 Gas *Chromatograph* - A gas **chromatograph** equipped with electron capture detector operated under the following suggested parameters. These parameters may be varied as per available facilities provided standardization is done.

Column	Glass, 1 meter long and 4.2 mm o.d. packed with 3 percent OV 17 on GO to 80 mesh gas chrom Q.
Carrier gas	Nitrogen flow rate 80 ml/min
Injection port temperature	250°C
Column oven temperature	240°C
Detector temperature	250°C

5.3 Reagent

5.3.1 Reference Standard of Phosalone (High Purity)

5.3.2 Prepare working standard solution of phosalone in acetone of 1.0 µg/ml by appropriate dilution of a more concentrated stock solution.

5.4 Procedure

5.4.1 Alkali Flame Ionization Detector (AFID)— Inject a range of duplicate volumes (2 to 5 µl) of the working standard solution into the column. Measure peak response as peak height/area. Calculate mass of pure phosalone from the volume of standard solutions injected. Now inject known volume (2 to 5 µl) of the **sample** extracts made to specific volume after partitioning/clean up. Measure the **peak** response of the sample at the specific retention time of the standard phosalone.

5.4.2 Electron Capture Detector (ECD).—Inject a volume of (2 to 8 μ l) of the working standard solution into the column. Measure peak response as height/area and calculate mass of phosalone from the volume injected. After standardization, inject known volume (2 to 8 μ l) of the sample, extract after clean up and made to specific volume. Measure peak response of the sample at specific retention time of the phosalone standard.

5.5 Calculations

$$\text{Phosalone residues, } \mu\text{g/g (ppm)} = \frac{A_s \times V_2 \times V_3 \times C}{A_s \times V_1 \times M} \times f$$

where

A_1 = peak height / area of the sample;

V_2 = volume, in μ l, of standard phosalone injected;

V_3 = total volume, in ml, of standard solution;

C = concentration, in μ g/g, of the standard solution;

$$f = \text{recovery factor} = \frac{100}{\text{percent mean recovery}}$$

A_2 = peak height / area of the phosalone standard;

V_1 = volume in μ l of sample injected; and

M = mass in g, of sample taken for analysis

NOTE 1 -Percent mean recovery is determined by raking untreated control sample to one in which known amount of phosalone are added and analysed as described above. Recoveries should be 80 percent or above.

NOTE 2 — The calculation can also be based on data obtained by the use of integrator, wherein the comparison of the area of the standard is made with that of sample.

INTERNATIONAL SYSTEM OF UNITS (SI UNITS)

Base Units

QUANTITY	UNIT	SYMBOL
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Thermodynamic temperature	kelvin	K
Luminous intensity	candela	cd
Amount of substance	mole	mol

Supplementary Units

QUANTITY	UNIT	SYMBOL
Plane angle	radian	rad
Solid angle	steradian	sr

Derived Units

QUANTITY	UNIT	SYMBOL	DEFINITION
Force	newton	N	$1 \text{ N} = 1 \text{ kg.m/s}^2$
Energy	joule	J	$1 \text{ J} = 1 \text{ N.m}$
Power	watt	W	$1 \text{ W} = 1 \text{ J/s}$
Flux	weber	Wb	$1 \text{ Wb} = 1 \text{ V.s}$
Flux density	terla	T	$1 \text{ T} = 1 \text{ Wb/m}^2$
Frequency	hertz	Hz	$1 \text{ Hz} = 1 \text{ c/s (s}^{-1}\text{)}$
Electric conductance	siemens	S	$1 \text{ S} = 1 \text{ A/V}$
Electromotive force	volt	V	$1 \text{ V} = 1 \text{ W/A}$
Pressure, stress	pascal	Pa	$1 \text{ Pa} = 1 \text{ N/m}^2$